Detection of serum antinuclear antibodies in lymphoma patients

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ABSTRACT. We investigated the presence of serum antinuclear antibodies (ANAs) and autoantibodies and their relationship with serum prognostic indicators in lymphoma patients. The study population comprised 127 patients diagnosed with lymphoma and 138 healthy control subjects. The blood samples of the participants were assayed for ANAs by immunofluorescence, and autoantibodies were detected by western blotting. Serum ANAs were detected in 31.5 (40/127) and 6.5% (9/138) of lymphoma patients and control subjects, respectively. There was a statistically significant difference between the lymphoma and the control groups ($P < 0.05$). The level of lactate dehydrogenase in the ANA-positive subjects was significantly lower than in the ANA-negative subjects ($P < 0.05$). Low ANA titers (1:100) were commonly found in the ANA-positive subjects and the control subjects, and the fluorescence models were diverse. Autoantibodies were found in 35% (14/40) of the ANA-positive patients by western blotting. Detection of ANAs in lymphoma patients helps in determining the diagnosis and prognosis of lymphoma, but has

no independent diagnostic value; there are still various autoantibodies of unknown significance that require further study.

Key words: Antinuclear antibodies; Autoantibodies; Lymphoma

INTRODUCTION

Antinuclear antibodies (ANAs) are a group of autoantibodies that target antigens of the nucleus in mammals. They are important serological markers of autoimmune disease. In recent years, increasing attention has been paid to the relationship between tumors and autoimmunity. Researchers have shown that ANAs relate not only to autoimmune diseases, but also to the majority of cancers, suggesting that they may be involved in the pathological processes of cancers and other diseases (Ma et al., 2010; Li et al., 2011). ANAs and autoantibodies in lymphoma patients were detected by immunofluorescence and western blotting to explore the clinical significance of ANAs in lymphoma.

MATERIAL AND METHODS

Subjects

Serum samples were collected from 127 patients diagnosed with malignant lymphoma (75 males and 52 females) in the First and Second Affiliated Hospitals of Xinjiang Medical University between 2005 and 2010. The study population comprised 86 Han patients, 36 Uygur patients, and 5 Kazakh patients, aged 6-87 years and with a median age of 56 years. According to the 2008 World Health Organization classification criteria for tumors of the hematopoietic and lymphoid system, the subjects comprised 14 cases of Hodgkin's lymphoma (HL) and 113 cases of non-Hodgkin's lymphoma (NHL) (including 14 cases of T-cell lymphoma and 99 cases of B-cell lymphoma). Healthy control subjects (138) without autoimmune diseases and of a similar age and gender to the patients group were also selected.

Methods

Venous blood (2 mL) was collected from each subject, and the serum was separated by centrifugation at 2500 g, for 10 min after natural coagulation. The serum was stored at -70°C. Serum ANA titer levels were detected by indirect immunofluorescence using Hep-2 (epithelial cell lines of human throat cancer) and monkey liver tissue sections as substrates, and fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG as secondary fluorescent antibodies, with a serum dilution of 1:100. A serum ANA-positive result was defined as the presence of specific green fluorescence in tissues or cells at a serum dilution of ≥ 1:100.

Western blotting

ANA-positive sera obtained by indirect immunofluorescence assay were used in the detection of autoantibodies by western blotting. Fifteen autoantibodies were detected: anti-snRNP/Sm, anti-Sm, anti-SSA, anti-SSB, anti-dsDNA, anti-histone, anti-ribosomal P protein, anti-Scl-70, anti-Jo-1, anti-Ro-52, anti-PCNA (anti-proliferating cell nuclear antigen antibody), anti-ACA (anti-

centromere antibody), anti-PM-Scl, anti-M2, and anti-nucleosome antibodies.

All the tests described above were conducted using a German EUROIMMUN kit (Berlin, German) in strict accordance with the manufacturer instructions for operation and interpretation of the experimental results.

Statistics

The count data are described by the number or percentage of cases. PEMS 3.1 statistical software was used to compare the rates using χ^2 analysis.

RESULTS

Relationship between ANA assay and lymphoma

Serum ANAs were detected in 31.5% (40/127) and 6.5% (9/138) of lymphoma patients and control subjects, respectively. There was a statistically significant difference between the positive rates of the lymphoma group and the control group ($P < 0.05$), but no significant difference between the HL and NHL groups, or the T-cell lymphoma and B-cell lymphoma groups ($P > 0.05$). There was no statistically significant difference in age, sex, or nationality ($P > 0.05$). The results are given in Table 1.

Table 1. ANA-positive rates in the different groups.

Group	N	No. of ANA-positive	Positive rate (%)
Control	138	9	6.5
Lymphoma	127	40	31.5
HL	14	5	35.7
NHL	113	35	30.9
T-cell lymphoma	14	3	21.4
B-cell lymphoma	99	32	32.3
Age			
≤50	52	15	28.8
>50	75	25	33.3
Gender			
Male	75	25	33.3
Female	52	15	28.8
Nationality			
Han	86	30	34.9
Uygur	36	9	25.0
Kazak	5	1	20.0

ANA = antinuclear antibody; HL = Hodgkin's lymphoma; NHL = non-Hodgkin's lymphoma. There were statistically significant differences between the ANA-positive rates in the lymphoma group and the control group.

The level of LDH in the ANA-positive subjects was significantly lower than in the ANA-negative subjects ($P < 0.05$), but there was no significant difference in the serum levels of β2-microglobulin (β-2MG) and C-reactive protein (CRP) between the two groups of subjects ($P > 0.05$) (Table 2).

ANA titer analysis and fluorescence models

ANA titer analysis showed that low ANA titers (1:100) were commonly found in ANA-positive subjects, accounting for 75% (30/40); titers of 1:320 accounted for 17.5% (7/40); and titers

of 1:1000 accounted for 7.5% (3/40). In the nine ANA-positive cases of the control group, there were seven cases with a titer of 1:100, one case with a titer of 1:320, and one case with a titer of 1:1000.

Further analysis of the ANA fluorescence models showed that the ANA fluorescence models of lymphoma patients were diversified, and nine different models were detected, of which the nucleolus model, the nuclear homogeneous model, and the nuclear particle model were the most common. Four kinds of fluorescence models were detected in the ANA-positive subjects in the control group, as shown in Table 3.

Table 2. Relationship between ANA and the serum levels of LDH, β-2MG, and CRP.

Group	N	x ± s	t-test	P value
LDH				
ANA(+)	38	210.9316 ± 83.9300	2.1282	P < 0.05
ANA(-)	77	297.4805 ± 336.2700		
β-2MG				
ANA(+)	27	4.8237 ± 5.6773	0.2865	P > 0.05
ANA(-)	48	4.4163 ± 6.3055		
CRP				
ANA(+)	22	28.9105 ± 41.7631	0.6586	P > 0.05
ANA(-)	40	22.421 ± 26.6877		

ANA = antinuclear antibody; LDH = lactate dehydrogenase; β-2MG = β2-microglobulin; CRP = C-reactive protein.

Table 3. Comparison of ANA fluorescence models in the different groupsANA fluorescence models.

	Control (N = 138)	Lymphoma (N = 127)
Nucleolus	5	14
Nuclear dots	1	0
Nuclear homogeneous	0	9
Nuclear particle	2	9
Cytoplasmic fiber	1	1
Spindle	0	4
Centrioles	0	3
Cytoplasmic granules	0	6
Golgi apparatus	0	3

ANA = antinuclear antibody.

Among the 40 cases of ANA-positive lymphoma subjects there were 32 cases with a single fluorescent model. Another eight cases had mixed-type fluorescent models; there were three cases of nuclear homogeneous and nuclear particle models, one case of nucleolar and cytoplasmic granules, one case of nucleolar and spindle, one case of nuclear particle and centrioles, one case of Golgi apparatus and spindle, and one case of nuclear particle, centrioles and cytoplasmic fiber.

Autoantibody spectrum assay

Forty serum samples of ANA-positive cases were further evaluated by western blotting. Serum autoantibodies were detected in 35% (14/40) of lymphoma patients and control subjects, with several positive bands (Table 4). Seven kinds of autoantibodies were found in the lymphoma patients, while only three kinds of autoantibodies were discovered in the control group.

Table 4. Western blotting of ANA-positive serum.							
Groups	SSA	SSB	Ro-52	M2	PM	Ds-DNA	Scl-70	RNP
Lymphoma	3	1	8	3	1	1	1	0
Control	1	0	1	0	0	0	0	3

ANA = antinuclear antibody.

DISCUSSION

Our results showed that in patients with malignant lymphoma, the ANA-positive rate was 31.5% - significantly higher than in the control group (6.5%, P < 0.05). Lymphoma often occurs in people with immune dysfunction. Some normal individuals also have autoantibodies, and the rate of occurrence increases with age (Li et al., 2011). Furthermore, tumor incidence is positively correlated to age. Therefore, it is still uncertain whether cancer patients with autoantibodies reflect cancer-relevance or age-relevance. However, the ANA-positive rate in our study was not related to age.

Research has shown that the immunogenicity of tumor cells can induce the production of a variety of autoantibodies: with SP2/0 tumor cell nuclei and normal DNA-immunized Balb/c mice, anti-dsDNA, anti-histone, anti-Sm, anti-ds-DNA, anti-ss-DNA, anti-histone, anti-Sm, anti-SS-A, and anti-SS-B were obtained (Shen et al.,2011). The experimental results showed that ANAs have anti-tumor activity. This may be mediated by antibody-dependent cell-mediated cytotoxicity (ADCC), by cytokines that enhance the immune function induced by ANA immune complexes, and by eliminating or reducing the inhibitory effect of extracellular chromatin on natural killer (NK) cell activity through the binding of ANAs and extracellular nuclear chromatin released from apoptotic tumor cells (Lu et al., 1998). Some cancer patients have been misdiagnosed in clinical situations owing to various serum autoantibodies and manifestations of rheumatism (Abu-Shakra et al., 2001; Solans-Laqué et al., 2004; Mu et al., 2006).

It has been reported that the mortality rate of cancer patients with autoimmune diseases is significantly lower than that of general cancer patients; the presence of circulating ANAs in lung cancer and colon cancer patients is related to prolonged disease progression and improved survival rate (Erkanli et al., 2006), but there are also contrary reports (Altintas et al., 2008). Autoimmune response is common in patients with lung cancer, and some asymptomatic patients may present with high levels of autoantibodies before the discovery of solid tumors or symptoms (Zhong et al., 2006; Murray et al., 2010).

The LDH level is a clinical prognosis indicator for patients with lymphoma, and high levels commonly correspond to poor prognosis (Shen and Wang, 2010). In this study, the ANA-negative subjects had higher LDH levels, suggesting that the prognosis for the ANA-negative subjects would be worse than for the positive subjects. This was consistent with the reported findings that ANA-positive patients with lung cancer and colon cancer have a prolonged disease progression and improved survival rate (Erkanli et al., 2006). This may be owing to the anti-tumor activity of ANAs mediating ADCC *in vivo*, the effects of cytokines, and the elimination or reduction of the inhibitory effect of extracellular chromatin on NK cell activity, as described above, as well as to other factors (Lu et al., 1998).

The results also showed that the ANA fluorescent models of the patients with malignant lymphoma were more diverse and abundant than those of the control group, and the spectrum of autoantibodies in the patient group was relatively wide. ANA titer analysis showed that ANA-positive lymphoma patients commonly had low titers (1:100), accounting for 75%, with few high titers. Our findings are consistent with previously reported results of ANAs in malignancies (Yang

et al., 2005), indicating that malignant lymphoma patients are subject to autoimmune phenomena, and such immune abnormalities and autoantibody overexpression may be another characteristic of lymphoma. Autoantibodies were detected in the sera of patients with lymphoma in the nucleolus, nuclear matter, cytoplasm, and many other sites. Autoantibodies were commonly detected in the nucleolus model (14 cases), the nuclear particle model (9 cases), and the nuclear homogeneous model (9 cases), suggesting that the targeted antigens for autoantibodies in lymphoma patients were mostly nucleolar antigens, ribonucleoproteins, histones, polynucleotides, and other proteins related to biosynthesis and cell proliferation. This study suggests that autoantibody detection will not only contribute to the diagnosis of patients with autoimmune diseases and healthy individuals, but also to the diagnosis of patients with lymphoma. Unlike previous domestic reports, in this study, mitosis-associated antigen antibodies were detected (spindle and central grain), and antibodies were also found in the Golgi apparatus. The clinical significance and diagnostic value of this discovery are unclear and require further research.

There were no significant difference between the ANA-positive rates in the HL and NHL groups, or between the T-cell lymphoma and B-cell lymphoma groups. Therefore, the detection of ANAs and the autoantibody spectrum contributes little to the diagnosis of different types of lymphoma, and needs to be combined with other clinical and laboratory markers.

In this study, 87 (68.5%) of the 127 lymphoma patients were found to be ANA-negative, and among the 40 ANA-positive patients, autoantibodies were detected in only 14 cases, suggesting that there may be other autoantibodies in lymphoma patients besides the common autoantibodies associated with autoimmune disease. This indicates that tumor cells may produce other antibodies besides ANAs and common autoantibodies through epitope expansion. Therefore, further research into the nature and biological effects of these autoantibodies will have a profound influence on the in-depth understanding of autoimmune lymphoma, as well as on its diagnosis, treatment, and prognosis.

Conflicts of interest

The authors declare no conflict of interest.

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